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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : Eisenberg
SERIAL NUMBER: 10/009,809 EXAMINER : Patrick J. Nolan
FILING DATE : April 26, 2002 ART UNIT : 1644
FOR : NOVEL ANTI-ALLERGIC AGENTS

DECLARATION OF RONIT SAGI-EISENBERG UNDER 37 C.F.R. §1.132

I, RONIT SAGI-EISENBERG, of 6 Lotus St., Nes-Ziona, Israel, declare and state that:

1. I am a coinventor, together with Tamar Raz, in the above-referenced patent application.

2. I received Ph.D. degree from Biochemistry, Tel Aviv University.

3. I am currently Associate Professor at the Department of Cell and Developmental Biology at the Sackler Faculty of Medicine at Tel Aviv University. I have more than 20 years of research experience in the fields of preclinical molecular biologic research as well as clinical research in allergy and have published more than 40 papers in top scientific journals. Prior to my current appointment, I was a visiting scientist at the National Institutes of Health, Bethesda MD and before that I held a position at the Weizmann Institute of Science.

4. I have reviewed the Office Action dated April 8, 2005. I understand that Claims 44-46 and 52-60 stand rejected under 35 U.S.C §103 as being unpatentable over Kuby et al. (herein referred to as "Kuby") in view of Aridor et al. (herein referred to as "Aridor") and U.S. Pat. No. 5,807,746 to Lin (hereafter referred to as "Lin").

5. I have reviewed the present application in conjunction with the Kuby, Aridor and Lin references.

6. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion that the pending claims are not obvious in view of the combination of Kuby, Aridor and Lin. The Examiner asserts that one of ordinary skill in the art would be motivated to add the importation peptides of Lin to the peptides taught by Aridor to treat allergies as treating

allergies with mast cell degranulator inhibitors was well known in the art as taught by Kuby and that one of ordinary skill in the art would have a reasonable expectation of success in producing the claimed invention . To the contrary, one of ordinary skill in the art would not have a reasonable expectation of success combining the teachings of Kuby, Aridor and Lin to reach the presently claimed invention.

7. The present invention as claimed is directed to methods for treating an allergic condition in a subject, by administering a pharmaceutically effective amount of a therapeutic agent to the subject, where therapeutic agent comprises a complex molecule having at least a first segment competent for importation of said molecule into mast cells *in vivo* (*i.e.*, AAVALLPAVLLALLAP), and a second segment for having an anti-allergic effect within said mast cells (*i.e.*, KENLKDCGLF or KNNLKECGLY), said first segment being joined to said second segment through a linker, whereby the complex molecule is capable of exerting its anti-allergic effect *in vivo*. As such, the claimed invention has two requirements: a) that the complex molecule (comprising an importation competent segment linked to an anti-allergic segment) be able to enter the cell *in vivo* and b) the complex molecule must be biologically active within the cell *in vivo* to exert its anti-allergic effect, thereby treating an allergic condition in a subject.

8. Lin and the instant specification show that the skilled artisan would not have a reasonable expectation of success in linking any importation signal peptide with any biological active molecule to allow the biological active molecule to be imported into a cell *in vivo* AND be biologically active to exert its therapeutic effect within the cell, *in vivo*. In fact, Lin discloses that, when compared to aFGF not linked to the importation peptide (k-FGF), the importation peptide kFGF linked to aFGF is less mitogenicically potent as shown by thymidine incorporation and DNA synthesis assays and the decreased mitogenic potency is significant, as aFGF not linked to an importation peptide at a concentration of 15 ng/ml stimulates DNA synthesis at a level more than twice that of the importation peptide comprising aFGF at 100 ug/ml (a concentration 10,000 fold greater than the concentration of aFGF).

9. The description in the present invention further demonstrates the unpredictability of linking any importation competent signal peptide with either KNNLKECGLY and KENLKDCGLF

to import the complex molecule into a cell, *in vivo*, such that the complex molecule retains its biological activity to exert its anti-allergic effect within the cell, *in vivo*. Specifically, six peptides comprising one of three importation competent signal peptides VTVLALGALAGVGVG (Human Integrin β_3 signal sequence), AAVALLPAVLLALLAP (Kaposi FGF signal sequence) and RQPKIWFPNRRKPWKK (*Antennapedia*, Drosophila transcription factor homeodomain signal sequence) linked to either KNNLKECGLY (C-terminus of $G\alpha_i$) or KENLKDCGLF (C-terminus of $G\alpha_t$) were synthesized. Based on the teachings of Aridor and Lin, as well as the Examiner's assertions, a skilled artisan would reasonably expect all six polypeptides to be imported into a mast cell *in vivo* and remain biologically active to exert its anti-allergic effect in the cell *in vivo* to treat an allergic condition in the subject. The Examiner's assertions are incorrect.

10. As is shown by the present invention, the biological activity (*i.e.*, anti-allergic effect) of these six peptides is highly divergent and unpredictable *in vitro* or *in vivo*. Specifically, the peptide VTVLALGALAGVGVGKNNLKECGLY (peptide 1, SEQ ID NO:6), was unable to exert an anti-allergic effect *in vitro* or *in vivo* as it failed to display any inhibitory activity. Whereas, the peptide RQPKIWFPNRRKPWKKKNNLKECGLY (peptide 3, SEQ ID NO:10), was not only was unable to exert an anti-allergic effect *in vitro* or *in vivo*, this peptide induced histamine secretion. Thus, this peptide acted in direct contradiction of what the ordinary skilled artisan would expect. Therefore, only two of the six peptides synthesized in the instant application: AAVALLPAVLLALLAPKENLKDCGLF (peptide 5, SEQ ID NO:12) and AAVALLPAVLLALLAPKNNLKECGLY (peptide 2, SEQ ID NO:7), were able to exert their anti-allergic effect in a cell *in vitro* or *in vivo* to treat an allergic condition in the subject.

11. I assert that the results of the present invention readily demonstrate that fusing a mast cell degranulation peptide as disclosed in Aridor to an importation sequence peptide as disclosed in Lin to treat an allergic condition as disclosed in Kuby does not predictable result in a peptide which can be imported into a mast cell *in vivo* and remain biologically active to exert its anti-allergic effect in the cell *in vivo* to treat an allergic condition in the subject as claimed by the present invention.

12. I also understand that Claim 52 stands rejected under 35 U.S.C §112, first paragraph as the Examiner alleges that mast cell degranulation is not recognized to be involved in causing pathology of multiple sclerosis (MS), and therefore, since there is no working example demonstrating the use of the peptides of the invention in treating MS, such use cannot be claimed.

13. I do not agree with the Examiner's assertion. Growing evidence suggests that mast cells (MCs) play a crucial role in the inflammatory process and the subsequent demyelination observed in patients suffering from MS. Zappulla J.P. et al. state that "Although no consensus exists on the role of mast cells in multiple sclerosis, recent results from animal models clearly indicate that these cells act at multiple levels to influence both the induction and the severity of disease. In addition to changing our views on the pathophysiology of multiple sclerosis, the concept that mast cells are critical for the outcome of the disease could have an important impact on the development of new therapeutic approaches."

14. Additionally, a recent review of Behi M.E. et al. shows number of evidences for the involvement of mast cells in MS. In central nervous system (CNS) of MS patients, increased mast cell population and activity have been documented. Ibrahim et al. have identified variable numbers of MCs both inside and around MS plaques. Moreover, the number of MCs around plaques was lower in acute lesions than in chronic active plaques, suggesting that the presence of MCs appears as a consequence of inflammation. Recently, the distribution of MCs in the brains of four MS patients showed that no MCs could be observed in healthy brains, while some MCs were present in MS plaques, essentially clustered around venules and capillaries. Another link between MCs and MS pathology is suggested by findings showing high concentrations of MC-released mediators, such as histamine and tryptase, an MC specific protease, in the cerebrospinal fluid of MS patients (Rozniecki J.J. et al). Several potential roles have been proposed for allergy mediators released by MCs in experimental autoimmune encephalomyelitis (EAE) and MS disease. One of these is the role of MC mediators in the breakdown of the blood brain barrier (BBB), an early and key event in the development of disease, opening the way to extravasation of inflammatory cells and molecules into the brain. As such, it has been shown that local alterations of BBB permeability after injection of the MC degranulator compound 48/80 (C40/80) into

experimental models have been occurred. MCs can also directly participate in the destruction of myelin in MS and EAE. MCs were shown to degranulate in response to major basic protein (MBP), leading to *in vitro* demyelination, and this process was essentially mediated by secreted proteases.

15. These findings, many available at the time of filing of the instant application, and the description of the role of substance-P induced neurogenic inflammation described in the specification at page 3, lines 11-18 highlight the importance of the role of MCs in MS. As such, I assert that one of ordinary skill the art would readily recognize that the modulation of mast cell functions using the complex molecules of the present can provide a novel therapeutic tool for the control of this human demyelinating disease and therefore, the specification and the state of the art at the time of filing, enables one of ordinary skill in the art to make and use the invention as claimed to treat multiple sclerosis.

16. I further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

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Attorney Docket No. 24025-501 NATL

Ronit Sagi-Eisenberg
Ronit Sagi-Eisenberg

Signed at Tel Aviv, ISRAEL
this 19 day of August, 2005

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